

Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry of Forensic Glass Samples

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ABSTRACT

Characterization of glass fragments is normally accomplished by measuring the physical and optical properties of density and refractive index. However further discrimination, such as identification of a suspected source, has become more difficult as the range of refractive indices has narrowed within glass subtypes because of advances in glass manufacturing technology. This project has evaluated laser ablation-inductively coupled plasma-mass spectrometry as an analysis technique, which can differentiate glass fragments of similar refractive indices based on the unique trace elemental signatures (or fingerprint) of the glass samples. Criteria and protocols for the comparison and differentiation of glass fragments from different sources, based on multivariate analysis techniques, have been developed. Additionally, the minimum amounts of glass sample needed to perform both qualitative and quantitative analyses have been determined.

PROJECT DESCRIPTION

The purpose of this project was to evaluate laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) as a technique the forensic examiner can use to uniquely identify and characterize, with a high level of certainty, glass trace evidence from a crime scene. LA-ICP-MS provides a means for the forensic examiner to assign a probability for positive association of a questioned glass sample based on its trace elemental composition. Knowing trace element concentrations improves the confidence of a match and

strengthens the value of evidence presented in court. ICP-MS provides a high level of discrimination for glass samples due to excellent detection limits (10-100 times better than ICP-atomic emission spectroscopy), wide element range coverage, and isotopic information. LA-ICP-MS is rapid, eliminates the need for extensive sample preparation, and is virtually a nondestructive technique (due to the extremely small amount of material consumed), allowing for the possibility of further analysis of questioned samples by corroborative techniques.

Furthermore, LA increases the number of analytically useful elements, compared to ICP-MS with sample dissolution and nebulization, by eliminating problems with some elements due to poor dissolution and contamination that may occur in an analysis from standard solution nebulization ICP-MS. A significant advantage of this type of analysis is that spectral comparison by Principal Component Analysis results in quantitative statistical comparison (yielding confidence intervals) without the need for elemental calibration or matrix-matched standards.

Additionally, smaller samples may be analyzed, making the technique applicable to more cases.

PROJECT OBJECTIVES

This project focused on standard residential window and tempered glass.

The objective of this project was to:

- 1) Study and determine the criteria for comparison and differentiation of glass samples provided by the Illinois State Police Sciences Command.

- 2) Determine the minimum sample size required for reproducible results.
- 3) Study the reproducibility on irregularly shaped glass surfaces.

PROCEDURES

ICP-MS

The mass spectrometer used in this study was a Thermo Finnigan Element 1 ICP-MS. This device employs magnetic and electrostatic analyzers configured in a reverse Nier-Johnson geometry to provide both mass and kinetic energy selection. The excellent detection limits (80 ppq aqueous ^{115}In), linear dynamic range ($>10^9$), sensitivity (5×10^9 cps ppm $^{-1}$ aqueous ^{115}In at low resolution), and stability (0.8% RSD for aqueous ^{115}In over 10 minutes) of this instrument are particularly favorable for the direct, ultra-trace analysis of solid fragments by laser ablation. For this study, the ICP-MS was operated in low resolution ($R = 300$).

The ICP load coil was “shielded” (CD-1 torch, Thermo Finnigan) to improve the ion transmission compared to a standard quartz ICP torch. With the shield grounded, the ICP is sustained only by inductive coupling, and the secondary discharge between the ICP and sampling cone is attenuated. Compared to analyses performed with a standard ICP torch, the shielded torch improves sensitivity by a factor of 5 to 20 (depending upon mass) while maintaining the extremely low background and high precision of the double-focusing instrument.¹

Laser Ablation

A CETAC Technologies LSX-100 was initially used to ablate the glass samples. This system consists of a Q-switched, frequency-quadrupled (266-nm) Nd:YAG laser and an ablation cell mounted on an xyz-translation stage. A charge-coupled device (CCD) camera with motorized zoom control permits real-time observation of the ablated site on a color monitor. The laser was operated at a maximum power of $3.6 \text{ mJ pulse}^{-1}$, with a repetition rate of 10 Hz. An in-house constructed argon fluoride (ArF) laser ablation system was also used in this study. This system consists of an MPB Technologies Inc., PSX-100 excimer laser. The ArF laser emits at 193 nm, with an average power of $4.0 \text{ mJ pulse}^{-1}$ at a repetition rate of 10 Hz. The ablation cell was mounted on a computer controlled xy-translation stage (Oriel, Inc.).

An argon flow rate of 1.3 L min^{-1} was used to transport the ablated particles to the plasma through a tygon tube approximately 1.5-m long x 3-mm internal diameter. For the sampling position and power used to operate the ICP, this gas flow rate maximized atomic ion signals for all the elements and samples measured.

Data Analysis for Comparison of Samples

A low-resolution full mass spectrum in the 4 to 240 mass range was acquired for each sample. Five spectra were acquired for each sample by ablating a raster pattern on the surface of a sample. The side of the glass

fragment selected for analysis was the non-“float” side, determined by using a handheld UV-lamp. The float side fluoresced when exposed to UV light. The full mass spectrum was used for analysis (i.e., no pre- or post- selection of isotopes was done).

Data preprocessing consisted of peak-area integration, background subtraction, and arrangement of the data into a form suitable for multivariate analysis. The data were saved as ASCII text and imported into Matlab v 6.1 (the MathWorks, Inc., Natick, MA) for PCA using PLS Toolbox 2.01f (Eigenvector Research, Manson, WA).

Principal component analysis for chemical and spectral analysis has been reviewed elsewhere.² Basically, it is a multivariate data reduction method that examines the variance patterns within a multidimensional data set. The dimensionality of the data set is reduced while retaining a major portion of the original information. This is accomplished by decomposing the correlation matrix of the variables of the data into a new set of axes, principal components, which define the directions of the major variances in the data set. The principal components are linear combinations of the variables (elements), comprised of three matrices that define each of the principal components: scores, loadings, and residuals. These matrices facilitate visualization of the relationships of the samples in the dataset and interpretation of the data. Scores describe the variance or relationship among the samples in the dataset and represent the contribution of the principal components in each sample. Loadings represent the contribution of the variables to the principal components and describe which

variables (or masses) are responsible for the variance in the data. The residuals represent random variations within the data set and are generally attributed to noise.

Samples within a particular group were compared by generating a PCA-model for the data from one sample. Spectra for other samples in the group were then compared to the model. The difference or variance of the sample spectra from the developed model was determined by the Q-statistic, which indicates how well each sample conforms to the model. The Q-statistic is simply the measure of the difference, or residual, between the mass spectrum from one sample and its projection into the PCA model created from the data for another sample. Confidence intervals for each of the samples within a particular group were calculated from the Q-statistic.

RESULTS AND DISCUSSION

Glass samples that could not be differentiated by refractive index were separated into groups. These groups are listed in Table I along with the density of the glass sample, if available. Two pieces of glass are said to be indistinguishable if their refractive indices are $N_D \pm 0.0002$, $N_F \pm 0.0004$, and $N_C \pm 0.0004$. Based on these refractive indices criteria, the glass samples within each of groups A, B, D, F, G, H, and I are indistinguishable. Within Groups C and E, some differentiation for some of the samples is possible. However, there are five possible pairs of glass samples in Group C that cannot be differentiated: 8 and 10, 8 and 1002, 10 and 1002, 8 and 11, 11 and 1002. If the density of

these samples were known, then all five pairs might possibly be differentiable. However, in typical casework, sample fragments are often not large enough to determine density. In group E there are nine possible pairs of glass samples that cannot be differentiated by refractive index: 4 and 1003, 4 and 1029, 1003 and 1029, 4 and 1013, 4 and 1036, 1013 and 1029, 1029 and 1036, 1001 and 1036.

Initial LA-ICP-MS studies of the glass samples were performed using the LSX-100. Examination of data sets showed large standard deviations in the ICP-MS signals for the 5 replicates acquired. Inspection of the acquired mass spectra revealed random spurious spikes in the various mass windows, indicative of large particles present in the ICP. Physical examination of the ablated glass samples showed a fair amount of fracturing/shattering within the samples themselves, which could possibly explain the presence of large particles in the ICP plasma. Previous ablation studies utilizing the LSX-100 system have shown that the system has poor laser beam quality (e.g., non-uniform energy distribution), which would account for the large standard deviations and sample fracturing. Remaining studies were conducted with the ArF laser and all of the PCA results discussed below were obtained using LA-ICP-MS data acquired using this system.

Principal component analysis was performed on all of the data sets and groups (26 glass samples separated into 9 groups). In all cases more than 99% of the variance within a group was accounted for in the first two principal components. The resulting score plots for all of the respective groups are shown in Figures 1 to 9. These score plots were generated from all of the data within a

particular group. Except for groups C and E, all of the plots show that the repetitions for the individual samples cluster together, but separate from other samples in score space. This separation indicates that the samples within those groups are different from the other members of the group, based on the acquired mass spectra (i.e., elemental composition).

Groups C and E, however, have some samples that overlap in score space when all of the samples within the group are used in the analysis. For example in group E, glass samples 1001, 1003, and 1036 appear to occupy the same area on the score plot, indicating that they are not distinguishable from one another in the PCA when all six of the samples are used in the model. There are several different approaches to further analyze the overlapping samples to determine if the samples are distinguishable from each another. The most direct approach, and the one used here, is to generate a PCA model from all of the repetitions for a sample and compare the other samples within the group to that model. As mentioned above, from the projection of the data onto the PCA model, one can ascertain the confidence interval. (The confidence interval is simply the probability of a sample being indistinguishable from another.) The confidence interval is calculated from the average Q-statistic for all of the repetitions.

Table II lists the calculated confidence intervals for the sample-to-sample comparisons for all of the groups. For example, consider the overlapping samples in the Group E score plot when sample 1001 is compared to the sample 1003 PCA model, 1001 has no better than 1 in 10^5 chance of being the same as

sample 1003. Similarly, sample 1003 has no better than a 1 in 10^7 chance of being the same as sample 1001 when sample 1003 is compared to the sample 1001 PCA model. The difference in the probabilities, although only two samples are being compared, arises from the scatter within several repetitions of the mass spectra.

Although not reported here, qualitative elemental differences between samples can also be ascertained from the principal component analysis. This information is contained in the loadings and represents the absence or relative difference of elements present in the sample-to-sample comparisons.

Quantitative elemental information can be obtained using LA-ICP-MS. The experimental procedure and data handling are outlined in Aeschliman *et al.*³

While quantitative elemental analysis is possible with LA-ICP-MS, a significant advantage of using PCA to compare samples is the elimination of the need for elemental calibration and matrix-matched standards. PCA is essentially a pattern-matching analysis and does not rely on calibration of the spectra to known concentrations. This reduces the need for expensive, hazardous, and costly materials and procedures for analysis. A second significant advantage is the elimination of bias due to pre-selection of elements. Since the analyst does not need to decide in advance which elements to calibrate, the full mass spectrum may be used, and the statistical comparison will determine which elements are significant for elimination or inclusion.

This study also investigated the effects of surface morphology on the ablation process and determined the minimum amount of glass sample required

for qualitative and quantitative analyses. Surface morphology presented little problem for laser ablation sampling of glass fragments. In all cases in this study, a sufficiently flat surface could be found on the fragments used. An advantage of laser ablation is the capability to perform small spatial sampling on samples on the order of the size of the laser beam diameter (10µm to 100µm diameter). Sampling is performed by repeatedly firing the laser on the same spot until the amount of desired data is collected. Comparison of data collected by rastering (moving the sample during sampling) and fixed spatial sampling for several of the glass sample groups showed little difference in differentiation of the samples by PCA.

In order to determine the minimum sample size required for reproducible analysis, the minimum numbers of laser shots for qualitative and quantitative analyses were determined. The appropriate number of laser shots was applied to a quartz microscope slide, and the dimensions of the resulting craters were measured. The general shape of the generated craters is conical. From the crater dimensions [radius (r) and depth (d)], simple geometry, and the density of the material, the volume and mass of the removed material is calculated [$V = \frac{1}{3}\pi r^2 d$]. These values are listed in Table III. It should be noted that the amount of material removed is dependent on the laser system used and sample properties. Different lasers might require more or fewer shots to remove the same amount of material, and different ICP-MS systems may be more or less sensitive.

DISSEMINATION DISCUSSION

The developed LA-ICP-MS technique and protocols from the project have already been used for actual casework by the MFRC (Sept. 2004 Vancouver, WA Police Department).

Preliminary results from the project have already been published.⁴ Additionally, the results from this project have been presented at the 2004 AAFS meeting in Dallas.⁵ An extensive case study touting the advantages of elemental analysis of glass fragments and the differentiating power of ICP-MS (without laser ablation) has recently been published by Montero et al.⁶ This paper parallels and corroborates the work and results in this project. Further dissemination of the project results will occur via this final project report, which will be distributed to all MFRC partners. The developed technique and protocols from the project will also be made available to all MFRC partners for casework assistance. Additionally we expect to publish a manuscript of this work in a peer-reviewed forensic journal.

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samples for analysis and performing the refractive index and density measurements.

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Table I. Glass Samples Undifferentiated By Refractive Index

<u>Group A</u>					
Sample 1010	Window	$N_D=1.5148$	$N_F=1.5212$	$N_C=1.5121$	-
Sample 1030	Tempered	$N_D=1.5149$	$N_F=1.5211$	$N_C=1.5123$	-
<u>Group B</u>					
Sample 1024	Window	$N_D=1.5155$	$N_F=1.5216$	$N_C=1.5130$	-
Sample 1032	Window	$N_D=1.5157$	$N_F=1.5221$	$N_C=1.5130$	-
<u>Group C</u>					
Sample 10	Window	$N_D=1.5169$	$N_F=1.5232$	$N_C=1.5143$	$D=2.4871$
Sample 8	Window	$N_D=1.5171$	$N_F=1.5234$	$N_C=1.5145$	$D=2.4936$
Sample 1002	Laminated	$N_D=1.5171$	$N_F=1.5235$	$N_C=1.5144$	$D=2.4909$
Sample 11	Tempered	$N_D=1.5172$	$N_F=1.5237$	$N_C=1.5145$	$D=2.4868$
<u>Group D</u>					
Sample 1035	Plate	$N_D=1.5175$	$N_F=1.5238$	$N_C=1.5148$	-
Sample 1033	Window	$N_D=1.5176$	$N_F=1.5240$	$N_C=1.5149$	$D=2.4946$
Sample 1009	Window	$N_D=1.5177$	$N_F=1.5239$	$N_C=1.5151$	$D=2.5016$
<u>Group E</u>					
Sample 1003	Tempered	$N_D=1.5183$	$N_F=1.5246$	$N_C=1.5156$	$D=2.4911$
Sample 4	Wire Reinforced	$N_D=1.5185$	$N_F=1.5247$	$N_C=1.5159$	$D=2.4940$
Sample 1029	Tempered	$N_D=1.5185$	$N_F=1.5250$	$N_C=1.5158$	-
Sample 1013	Window	$N_D=1.5186$	$N_F=1.5249$	$N_C=1.5159$	$D=2.4939$
Sample 1036	Tempered	$N_D=1.5187$	$N_F=1.5251$	$N_C=1.5159$	-
Sample 1001	Tempered	$N_D=1.5189$	$N_F=1.5255$	$N_C=1.5161$	$D=2.4942$
<u>Group F</u>					
Sample 1	Gray Window	$N_D=1.5196$	$N_F=1.5259$	$N_C=1.5170$	$D=2.4975$
Sample 1017	Tempered	$N_D=1.5197$	$N_F=1.5263$	$N_C=1.5169$	$D=2.4964$
<u>Group G</u>					
Sample 14	Wire Reinforced	$N_D=1.5127$	$N_F=1.5292$	$N_C=1.5199$	-
Sample 16	Window	$N_D=1.5129$	$N_F=1.5294$	$N_C=1.5201$	-
<u>Group H</u>					
Sample 1006	Window	$N_D=1.5138$	$N_F=1.5302$	$N_C=1.5210$	-
Sample 1034	Dark Gray	$N_D=1.5138$	$N_F=1.5302$	$N_C=1.5210$	-
<u>Group I</u>					
Sample 18	Patterned	$N_D=1.5147$	$N_F=1.5313$	$N_C=1.5219$	$D=2.5250$
Sample 17	Window	$N_D=1.5148$	$N_F=1.5311$	$N_C=1.5221$	$D=2.5139$
Sample 19	Plate	$N_D=1.5149$	$N_F=1.5314$	$N_C=1.5221$	$D=2.5210$
Differentiation Criteria		$N_D \pm 0.0002$	$N_F \pm 0.0004$	$N_C \pm 0.0004$	

Table II. Probability of Sample Being Same as Model

<u>Group A</u>						
	s1010	s1030				
Model 1010	-	$< 10^{-14}$				
Model 1030	$< 10^{-14}$	-				
<u>Group B</u>						
	s1024	s1032				
Model 1024	-	$< 10^{-14}$				
Model 1032	$< 10^{-14}$	-				
<u>Group C</u>						
	S8	s10	s11	s1002		
Model 8	-	$< 10^{-14}$	$< 10^{-14}$	$< 10^{-14}$		
Model 10	7×10^{-11}	-	$< 10^{-14}$	$< 10^{-14}$		
Model 11	$< 10^{-14}$	$< 10^{-14}$	-	$< 10^{-14}$		
Model 1002	1×10^{-07}	3×10^{-04}	$< 10^{-14}$	-		
<u>Group D</u>						
	S1009	S1033	S1035			
Model 1009	-	7×10^{-11}	$< 10^{-14}$			
Model 1033	$< 10^{-14}$	-	$< 10^{-14}$			
Model 1035	8×10^{-07}	3×10^{-08}	-			
<u>Group E</u>						
	S4	s1001	s1003	s1013	s1029	s1036
Model 4	-	$< 10^{-14}$	$< 10^{-14}$	$< 10^{-14}$	5×10^{-04}	$< 10^{-14}$
Model 1001	$< 10^{-14}$	-	5×10^{-07}	5×10^{-12}	5×10^{-13}	2×10^{-06}
Model 1003	$< 10^{-14}$	3×10^{-05}	-	1×10^{-12}	2×10^{-11}	6×10^{-04}
Model 1013	$< 10^{-14}$	$< 10^{-14}$	$< 10^{-14}$	-	$< 10^{-14}$	$< 10^{-14}$
Model 1029	8×10^{-07}	$< 10^{-14}$	$< 10^{-14}$	$< 10^{-14}$	-	$< 10^{-14}$
Model 1036	$< 10^{-14}$	9×10^{-12}	2×10^{-07}	$< 10^{-14}$	1×10^{-11}	-
<u>Group F</u>						
	s1	s1017				
Model 1	-	5×10^{-09}				
Model 1017	2×10^{-12}	-				
<u>Group G</u>						
	s14	s16				
Model 14	-	$< 10^{-14}$				
Model 16	3×10^{-07}	-				
<u>Group H</u>						
	S1006	s1034				
Model 1006	-	7×10^{-07}				
Model 1034	$< 10^{-14}$	-				
<u>Group I</u>						
	s17	s18	s19			
Model 17	-	$< 10^{-14}$	$< 10^{-14}$			
Model 18	$< 10^{-14}$	-	$< 10^{-14}$			
Model 19	9×10^{-07}	$< 10^{-14}$	-			

Table III. Amount of Material Removed.

	<u>Qualitative Comparison</u>	<u>Quantitative Analysis</u>
Number of Shots	570	250
Crater Diameter	$87 \pm 0.58 \mu\text{m}$	$105 \pm 0.5 \mu\text{m}$
Crater Depth	$180.8 \pm 3.2 \mu\text{m}$	$122.3 \pm 2.5 \mu\text{m}$
Amount of Material Removed*	788 ng	776 ng

* Density of Fused Silica Glass= 2.2 g/cc

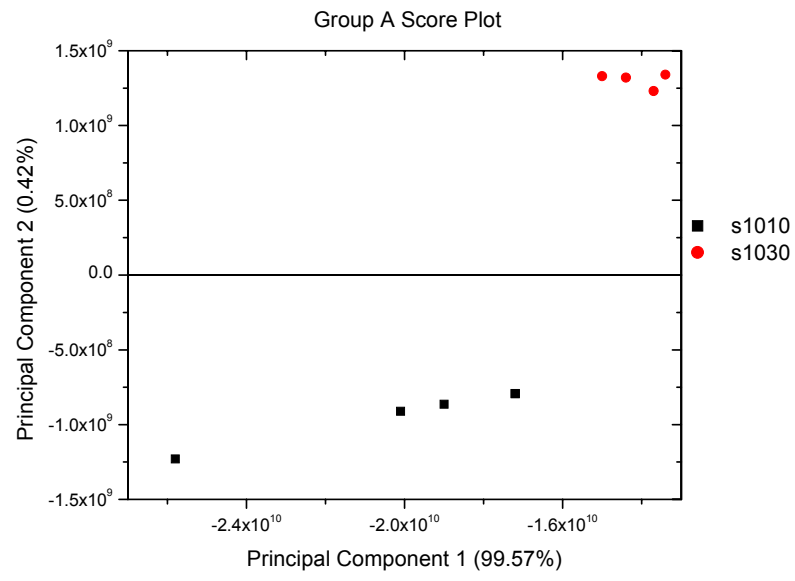


Figure 1. PCA score plot for glass samples in Group A.

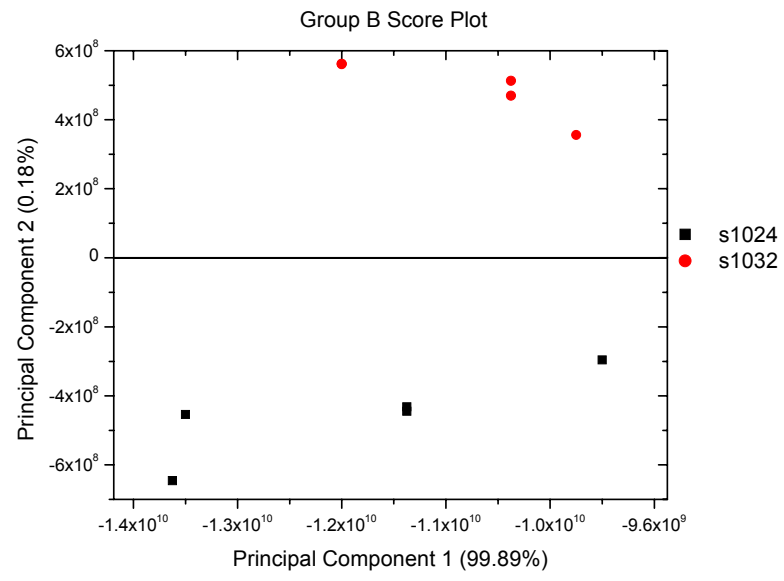


Figure 2. PCA score plot for glass samples in Group B.

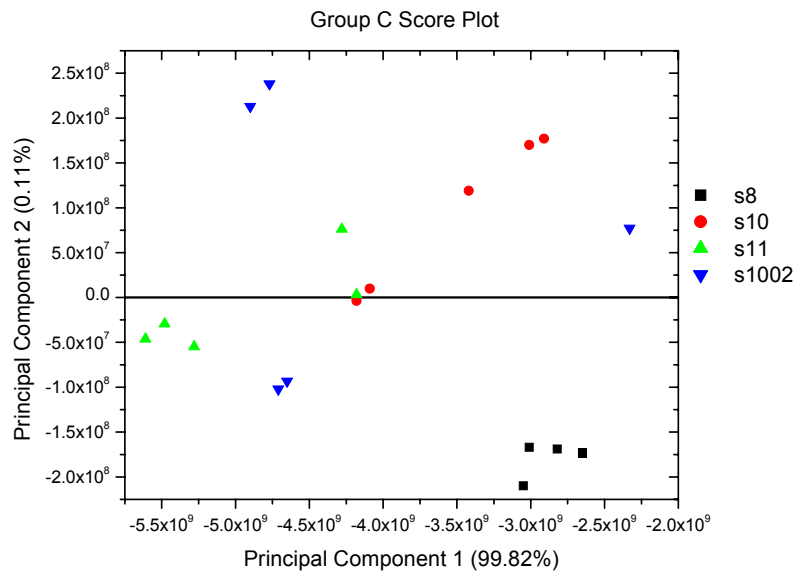


Figure 3. PCA score plot for glass samples in Group C.

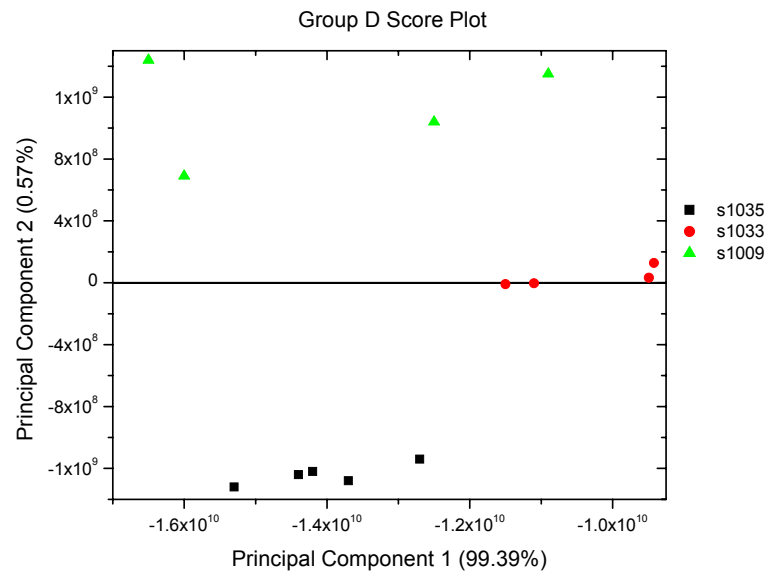


Figure 4. PCA score plot for glass samples in Group D.

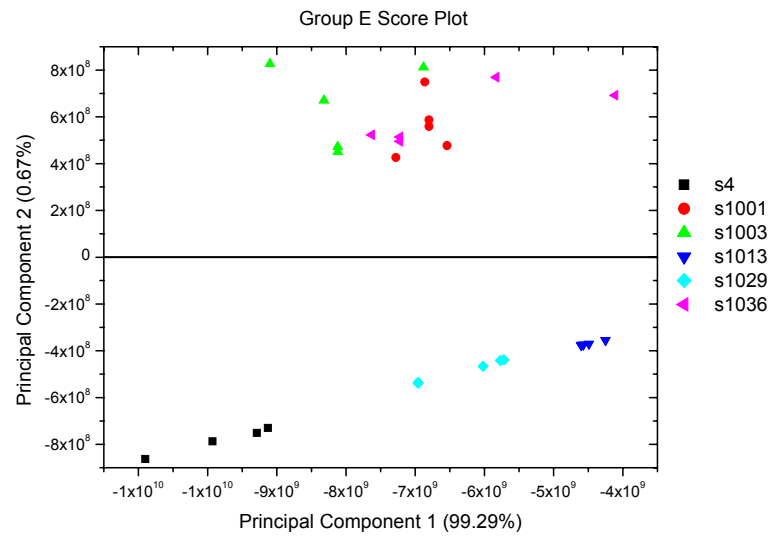


Figure 5. PCA score plot for glass samples in Group E.

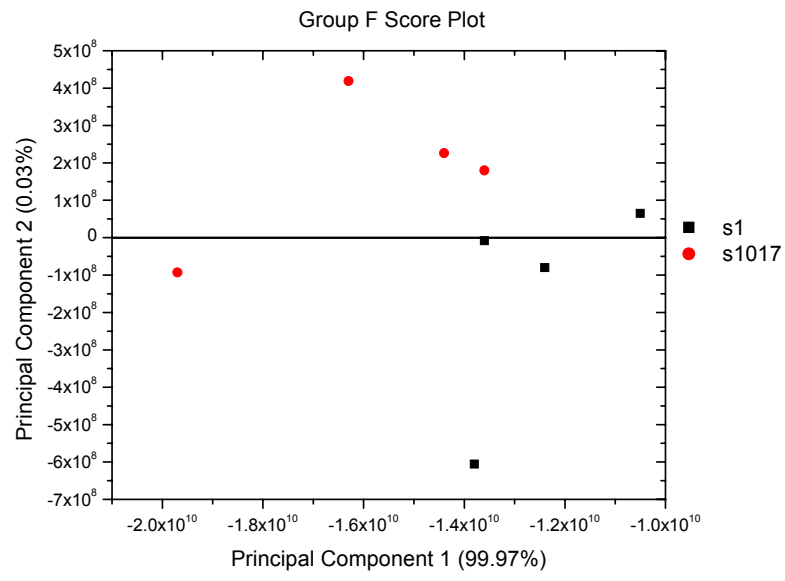


Figure 6. PCA score plot for glass samples in Group F.

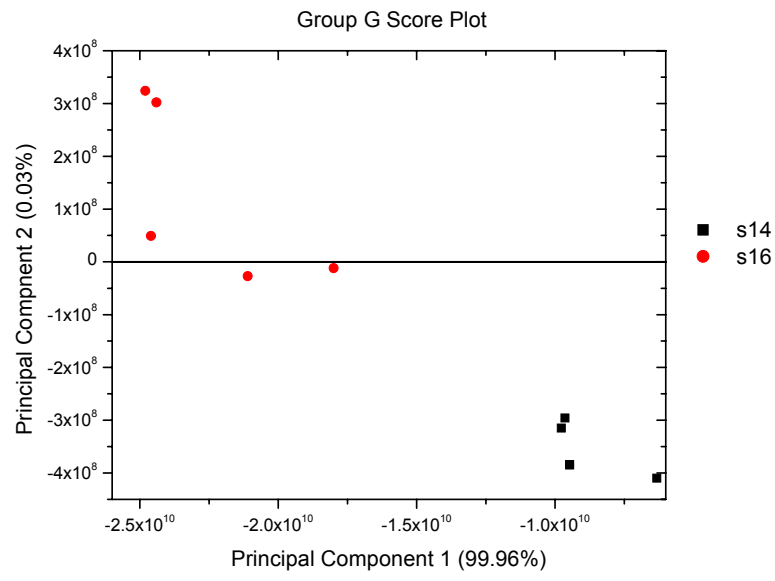


Figure 7. PCA score plot for glass samples in Group G.

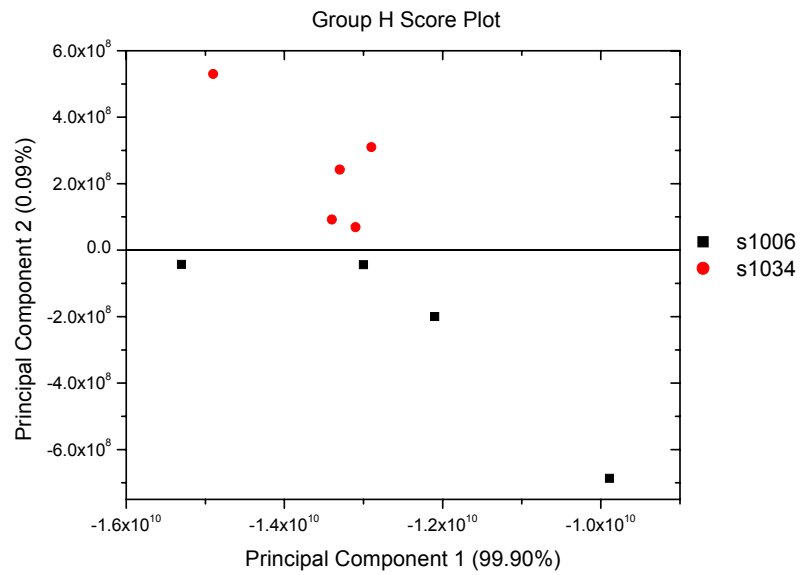


Figure 8. PCA score plot for glass samples in Group H.

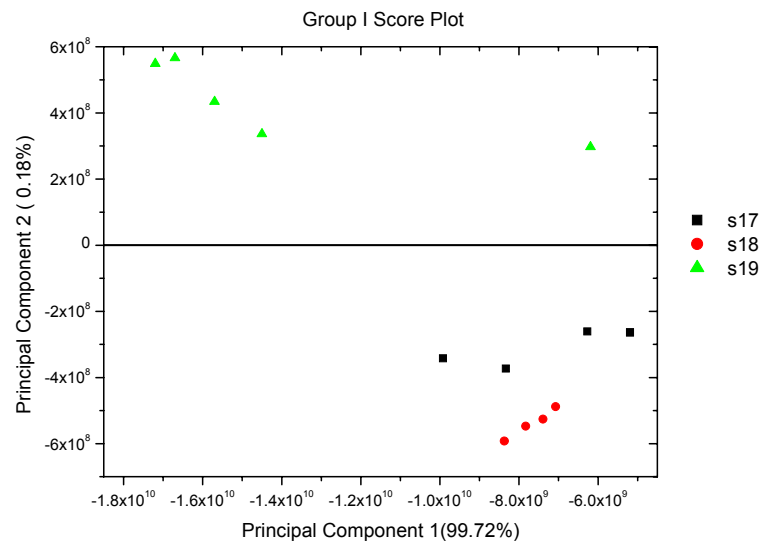


Figure 9. PCA score plot for glass samples in Group I.